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Human Genome Epidemiology (HuGE) Review

Factor XIII Val34Leu Variant Is Protective against Venous Thromboembolism: A HuGE Review and Meta-Analysis

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It has been suggested that a *G*-to-*T* transition in exon 2 of the *factor XIIIA* gene resulting in a substitution of leucine for valine at amino acid 34 (*FXIII Val34Leu*) protects against venous thromboembolism (VTE). However, the evidence to date is insufficient to incorporate testing for the *FXIII Val34Leu* variant into clinical practice. To determine whether genotypes with the *FXIII Val34Leu* variant are protective against VTE, the authors performed a meta-analysis of 12 studies with genotyping for the *FXIII Val34Leu* variant (3,165 objectively diagnosed VTE cases and 4,909 controls). When a random-effects model was used, the combined odds ratios for VTE were 0.63 (95% confidence interval: 0.46, 0.86) for the homozygotes of the *FXIII Val34Leu* variant, 0.89 (95% confidence interval: 0.80, 0.99) for the heterozygotes, and 0.85 (95% confidence interval: 0.77, 0.95) for the homozygotes and heterozygotes combined. Potential sources of heterogeneity and potential bias were explored. The meta-analysis provided evidence that the *FXIII Val34Leu* variant has a small, but significant protective effect against VTE. Since VTE is a complex disorder, this information, along with results of ongoing studies to identify additional genetic factors underlying VTE, will be crucial in developing accurate risk profiles to identify individuals at higher risk of VTE.

epidemiology; factor XIII; *FXIII Val34Leu*; genetics; leucine; meta-analysis; valine; venous thrombosis

Abbreviations: CI, confidence interval; FXIII, factor XIII; FXIIIA, factor XIII A subunit; *FXIII Val34Leu*, *factor XIII Valine34Leucine* variant; Leu, leucine; Val, valine; VTE, venous thromboembolism.

Editor's note: *This paper is also available on the website of the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/).*

GENE

Factor XIII (FXIII), also called fibrin stabilizing factor, has a crucial role in the blood coagulation and fibrinolytic pathways. Plasma FXIII is an inactive enzyme precursor that circulates in plasma in the form of two pairs of non-identical A and B subunits. The gene encoding for the B subunit of FXIII has been assigned to chromosome 1q31-32 (1) and helps stabilize and transport the A subunit in plasma (2). The gene coding for the A subunit of FXIII (*FXIIIA*) is localized on chromosome 6p24-25. FXIIIA exhibits the transglutaminase activity (i.e., the activated form of FXIII), which, along with thrombin-activated fibrinolysis inhibitor, is involved in stabilizing the fibrin clot and making the clot more lysis resistant (3).

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Mutations in the *FXIIIA* gene have been associated with FXIII deficiency, which in turn is associated with the tendency toward spontaneous bleeding and several related morbid events (4, 5). Despite being associated with severe bleeding in some cases, several of these mutations have little impact at the population level because they are rare (4, 6). The *FXIII Val34Leu* variant is common, however, and has also been associated with higher transglutaminase activity, leading to decreased clot formation (7–10).

GENE VARIANT

The *FXIII Val34Leu* variant is a *G*-to-*T* transition in exon 2 of the gene encoding for FXIIIA, leading to a valine (Val)-to-leucine (Leu) substitution at amino acid 34 (5). This variant is common in White populations, with a frequency of approximately 0.25–0.30 (11–13). However, the frequency varies among ethnic groups, with the lowest (0.01) in Japanese and the highest (0.40) in Pima Indians (2).

The biochemical consequences of the *FXIII Val34Leu* variant are not well understood. The *FXIII Val34Leu* variant does not result in a change in the plasma concentration of FXIII, but the amino acid change may modify FXIII activity (7, 9, 14, 15). Activation of FXIIIA by thrombin was found to proceed two- to threefold more rapidly in plasma of *FXIII Val34Leu* variant carriers (8–10, 16). This action has an effect on clot stability since the catalytic efficiency of thrombin-induced cleavage of FXIIIA alters the structure of the cross-linked fibrin such that fibrin fully cross-linked by *FXIII Val34Leu* product has a finer structure with thinner fibers and smaller pores. Lateral aggregation of fibrin fibers is impaired (17).

DISEASE

A disruption in normal hemostasis that maintains blood fluidity and prevents blood loss could result in the formation and growth of thrombi that can obstruct venous circulation and may embolize. These common diseases are referred to as deep vein thrombosis and pulmonary embolism, collectively known as venous thromboembolism (VTE). Clinical implications of VTE include morbidity from pain, swelling, or dyspnea from pulmonary embolism; bleeding risk from anticoagulant treatment to prevent recurrent events; postphlebitic syndrome; and death from pulmonary embolism, which, in many cases, is sudden. Although a number of acquired and inherited predisposing factors are known, we cannot accurately identify which persons will experience a VTE. Accurate identification of patients at risk would be of great clinical utility since one third of cases of VTE are the more serious pulmonary emboli and approximately 22 percent of patients with pulmonary embolism die before being diagnosed (18–20).

Inherited thrombophilia is the term applied to the genetic predisposition to VTE. Thrombophilia is a highly prevalent problem, with more than 10 percent of the population affected by one of the currently known genetic risk factors, the common ones being *factor V Leiden* and *Prothrombin G20210A*, and it is identified in at least 50 percent of cases of VTE (21). However, approximately 50 percent of familial cases are unexplained despite accounting for known genetic or acquired thrombophilias. Furthermore, many patients with inherited thrombophilia never develop a VTE, suggesting the existence of protective factors. Thus, the mechanisms underlying VTE are still largely unknown, so the individual risk profile remains largely unpredictable.

Several case-control studies have suggested a protective effect of the *FXIII Val34Leu* variant against VTE (22–25). However, most studies had sample sizes insufficient to detect statistically significant differences, while a few others reported no effect of this variant on the risk of VTE (9, 26, 27). Variants with a protective effect are of interest because they provide insight into the biochemical pathways conferring the beneficial effect against VTE. Identifying such variants is also useful in establishing risk profiles to identify those at risk of developing VTE.

We conducted a meta-analysis to determine whether genotypes with the *FXIII Val34Leu* variant confer protection against VTE. Results from this meta-analysis suggest that incorporation of the *FXIII Val34Leu* variant will be useful in developing risk-profiling tools to assess the risk of VTE.

META-ANALYSIS METHODS

Identification and eligibility of relevant studies

We considered all studies (published in full or in abstract form) that examined the association between the *FXIII Val34Leu* variant and VTE. Electronic databases were searched by using the OVID search engine (Ovid Technologies, Inc., New York, New York) for MEDLINE (National Library of Medicine, Bethesda, Maryland) (1966 to week 2 of June 2004) and EMBASE (Elsevier B.V., the Netherlands). The search strategy was based on combinations of the terms ''venous thromboembolism,'' ''factor XIII,'' ''valine,'' and ''leucine.'' This electronic search was followed by a manual search of reference lists from retrieved articles, symposia proceedings, and abstracts from major thrombosis conferences. The search was not limited to the English language.

Studies were included if the distribution of genotypes for the *FXIII Val34Leu* variant was reported for patients with objectively diagnosed VTE and for a control population (i.e., without VTE). Objective diagnosis of VTE was based on results of ultrasonography, phlebography, high-probability ventilation-perfusion lung scan with a moderate-to-high pretest clinical probability, spiral computed tomographic scan with contrast medium, or pulmonary angiography.

Data extraction

Two examiners independently extracted data from the studies by using a standardized form. Any disagreements were resolved by discussion or in consultation with a third examiner. The collected information from each report included the authors, journal and year of publication, country of origin, selection and characteristics of VTE cases and controls, demographics, ethnic group of the study

population, and number of cases and controls for each *FXIII* genotype.

Meta-analysis

Prior to pooling the studies for the meta-analysis, Hardy-Weinberg equilibrium was assessed in the control groups of individual studies by using the goodness-of-fit χ^2 statistic with one degree of freedom. A two-sided p value of >0.05 was considered consistent with Hardy-Weinberg equilibrium. The choice of the comparison groups for the meta-analysis was based on the hypothesis that the *Leu* allele (i.e., *factor XIII Val34Leu*) has a protective effect against VTE. Therefore, we performed three comparisons, with the *Val/Val* genotype as the reference group: *Leu/Leu* genotype, *Leu/Val* genotype, and both groups together (*Leu/Leu* + *Leu/Val* combined). We used the odds ratio with a 95 percent confidence interval as the metric of risk.

For each comparison, we tested the between-study heterogeneity by using the Breslow-Day χ^2 statistic (28). Because tests for heterogeneity may have low power when the sample sizes are small, as in several of the studies to be included in our meta-analysis, we chose to be conservative by using a two-sided p value of <0.10 as the threshold for considering the test significant for heterogeneity, as suggested by Attia et al. (29). We also used the I^2 statistic, which describes the percentage of variability in point estimates due to sample heterogeneity rather than sampling error (30, 31). I^2 values from 31 percent to 56 percent have been defined as indicating ''low'' to ''moderate'' heterogeneity, whereas values greater than 56 percent are considered indicative of ''notable'' heterogeneity (31). We chose to use the DerSimonian-Laird random-effects model (32) to combine the data because the selection criteria were not entirely identical among the eligible studies and because heterogeneity tests may lack power in some contexts. This approach incorporates an estimate of the between-study variance; therefore, the confidence intervals tend to be wider when the studies differ among themselves. Again, this approach leads to more conservative estimates (33).

Because the frequency of the *FXIII Val34Leu* variant differs substantially across ethnic groups (2), and because the large majority of the study subjects were White, we also performed a subgroup analysis that excluded the two studies that clearly enrolled non-Whites (23, 34), and, for one paper, we included the data generated for Whites only (35). In studies that did not describe the ethnicity of the population clearly, we contacted the authors. Furthermore, two studies ascertained their cases quite differently from the other studies; for example, the cases were further ascertained for known thrombophilias (9, 27), and, in one study, the genotype distribution in the control group was not in Hardy-Weinberg equilibrium (23). To verify whether this affected the results, we reanalyzed the data by also excluding these studies. Funnel plots were generated to assess publication bias, particularly whether large studies lead to different results than smaller studies (36). Statistical analysis was performed with SAS software (version 8.2; SAS Institute, Inc., Cary, North Carolina).

META-ANALYSIS RESULTS

Eligible studies

Characteristics of the 12 studies included in the meta-analysis (9, 17, 22–27, 34, 35, and 37; P. Wells, University of Ottawa, unpublished manuscript) are shown in table 1. The studies were conducted on three continents: Europe, North America, and South America; six studies were reported to be multicenter. More than 80 percent of the subjects in each individual study were White. These studies were not entirely uniform with respect to case and control selection. Five studies were limited to cases with isolated deep vein thrombosis (9, 17, 23, 26, 34), and seven studies included cases with deep vein thrombosis and/or pulmonary embolism (22, 24, 25, 27, 35, and 37; P. Wells, unpublished manuscript). Only one study limited cases to idiopathic VTE (P. Wells, unpublished manuscript), while an additional three studies excluded patients with malignancy (9, 23, 34). Six studies used upper age limits in the selection of cases; these studies included only those patients with VTE prior to age 45 years (9) or patients less than age 70 years (17, 34, 35). Compared with the rest of the studies, those by Balogh et al. (9) and Margaglione et al. (27) are the most different with regard to the ascertainment of cases because they focused on groups that may have been ''extreme'' VTE cases.

Meta-analysis database

The distribution of the *FXIII* genotypes among VTE cases and controls in the eligible studies is presented in table 2. A total of 8,074 subjects with genotype data were available, with 3,165 VTE cases and 4,909 controls. The distribution of genotypes in control groups was consistent with Hardy-Weinberg equilibrium in 11 of the 12 studies. The control group genotypes in the Franco et al. (23) study were not in Hardy-Weinberg equilibrium, with $\chi^2 = 6.42$ and $p < 0.02$.

Overall effects

Our results suggest that the *FXIII Val34Leu* variant confers a small, but significant protective effect against VTE (figures 1–3). When a random-effects model was used, the *Val/Val* genotype being the reference group, the combined odds ratios for VTE were 0.63 (95 percent confidence interval (CI): 0.46, 0.86) for the homozygotes (*Leu/Leu* vs. *Val/Val*) (figure 1), 0.89 (95 percent CI: 0.80, 0.99) for the heterozygotes (*Leu/Val* vs. *Val/Val*) (figure 2), and 0.85 (95 percent CI: 0.77, 0.95) for *Leu/Val* and *Leu/Leu* (figure 3) vs. *Val/Val*. There was no statistically significant between-study heterogeneity for any of the comparison groups as assessed by the Breslow-Day test, with p values of >0.4. The I^2 statistic was consistent with these results, suggesting only ''very low'' (10 percent) to ''moderate'' heterogeneity.

Bias diagnostics

Removing the two studies that clearly enrolled non-Whites (23, 34) did not affect the results. The odds ratios remained consistent with a protective effect against VTE

TABLE 1. Characteristics of studies included in the meta-analysis

First author, year (reference no.)	Country	Mean age (years)		Subjects (no.)		Selection	
		Cases	Controls	Cases	Controls	Cases	Controls
Catto, 1999 (22)	United Kingdom	62.0*	60.0*	217	252	Clinical VTE† diagnosis	No personal or family history of VTE
Franco, 1999 (23)	Brazil	41.0	41.0	189	187	Aged <65 years at first DVT† episode	Unrelated; asymptomatic; healthy; no history of VTE
Corral, 2000 (26)	Spain	60.9	60.8	97	97	Confirmed diagnosis of DVT	No history of vascular disease
Renner, 2000 (25)	Austria	53.2	53.2	154	308	Admitted with a documented DVT	No history of VTE or arterial disease
Balogh, 2000 (9)	Hungary	35.2	30.3	273	288	Patients with VTE before age 45 years; family history of VTE or recurrent thrombosis or unusual location	Healthy volunteers; no personal or family history of VTE, arterial disease, or malignancy
Margaglione, 2000 (27)	Italy	45.0*	36.0*	427	1,045	Referred for thrombophilia workup; DVT and PE† diagnosed objectively	Healthy; no clinical history of VTE
Alhenc-Gelas, 2000 (24)	France	42.6	42.7	354	1,229	Aged <61 years; objectively diagnosed DVT and/or PE	No history of VTE, arterial disease, or malignancy
van Hylckama Vlieg, 2002 (17)	The Netherlands	N/R†	N/R	471	474	Aged <70 years; referred for anticoagulation treatment; first objectively diagnosed DVT	No history of VTE or malignancy; no use of OACs† for at least the prior 3 months; same geographic area
Dowling, 2003 (35)	United States	49.2‡	49.5‡	190	157	Aged 18–70 years; hospitalized for VTE	No history of VTE, mental/physical problems, or anticoagulant use
Zidane, 2003 (37)	The Netherlands	54.9	47.4	66	148	Objectively confirmed PE	Suspected PE confirmed as absent (internal control group)
Wells, 2004 (unpublished manuscript)	Canada	56.2	56.4	309	306	Objectively confirmed, idiopathic VTE	Healthy friends; no history of VTE, malignancy, or use of OACs for at least the prior 3 months
Pintao, 2004 (34)	Brazil	42.0	42.0	418	418	Aged <70 years; no history of malignancy; objectively diagnosed DVT	Healthy blood donors; no history of VTE, arterial thrombosis, or malignancy

* Median age.
† VTE, venous thromboembolism; DVT, deep vein thrombosis; PE, pulmonary embolism; N/R, not reported; OACs, oral anticoagulants.
‡ Mean age reported for the total population.

associated with the *FXIII Val34Leu* variant. Compared with those for the *Val*/*Val* genotype group, the odds ratios were 0.75 (95 percent CI: 0.58, 0.97) for the *Leu*/*Leu* genotype group, 0.88 (95 percent CI: 0.78, 0.99) for the *Leu*/*Val* genotype group, and 0.86 (95 percent CI: 0.76, 0.98) for the combined *Leu*/*Leu* and *Leu*/*Val* genotype groups. Between-study homogeneity remained when these two studies were excluded from the meta-analysis. Finally, reanalysis of the data excluding the two studies mentioned above (23, 34) and the two studies that ascertained cases by using either very low upper age limits and/or persons with known thrombophilia (9, 27), or those studies not in Hardy-Weinberg equilibrium (23), did not change our conclusion. Again, the odds ratios remained consistent with a protective effect against VTE associated with the *FXIII Val34Leu* variant. Compared with those for the *Val*/*Val* genotype group, the odds ratios were 0.61 (95 percent CI: 0.46, 0.80) for the *Leu*/*Leu* genotype group, 0.84 (95 percent CI: 0.72, 0.97) for the *Leu*/*Val* genotype group, and 0.80 (95 percent CI: 0.71, 0.92) for the combined *Leu*/*Leu* and *Leu*/*Val* genotype groups. Between-study homogeneity remained when these studies were excluded from the meta-analysis, with the I^2 statistic much below 30 percent. Publication bias was also unlikely as demonstrated by the funnel plot analysis, which showed symmetric odds ratios against study sample size (data not shown).

DISCUSSION

To our knowledge, our meta-analysis of 12 studies, involving over 3,000 genotyped cases and about 5,000 controls, provides the most comprehensive assessment so far of the association of the *FXIII Val34Leu* variant with VTE. It

TABLE 2. Distribution of the *factor XIII* genotypes* among venous thromboembolism cases and controls in the included studies

First author, year (reference)	*Val/Val*				*Val/Leu*				*Leu/Leu*			
	Cases		Controls		Cases		Controls		Cases		Controls	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Catto, 1999 (22)	137	29	123	26	68	15	107	23	12	2	22	5
Franco, 1999 (23)	116	31	110	29	70	18	59	16	3	1	18	5
Corral, 2000 (26)	61	31	66	34	34	18	28	14	2	1	3	2
Renner, 2000 (25)	97	21	163	35	49	11	121	26	8	2	24	5
Balogh, 2000 (9)	144	26	158	28	111	20	111	20	18	3	19	3
Margaglione, 2000 (27)	276	19	677	46	123	8	319	22	28	2	49	3
Alhenc-Gelas, 2000 (24)	225	14	728	46	115	7	437	28	14	1	64	4
van Hylckama Vlieg, 2002 (17)	286	30	273	29	165	18	174	18	20	2	27	3
Dowling, 2003 (35)	111	32	86	25	69	20	53	15	10	3	18	5
Zidane, 2003 (37)	36	17	80	37	27	13	55	26	3	1	13	6
Wells, 2004 (unpublished manuscript)	184	30	164	27	110	18	122	20	15	2	20	3
Pintao, 2004 (34)	261	31	236	28	148	18	157	19	9	1	25	3
Total	1,934		2,864		1,089		1,743		142		302	

* *Val*, valine; *Leu*, leucine.

supplies evidence for a protective effect of the *FXIII Val34-Leu* variant against VTE in both homozygotes and heterozygotes for the variant, with significantly lower odds ratios. The protective effect was stronger for the homozygotes alone than for the heterozygotes alone or combined with the homozygotes. This conclusion was further supported

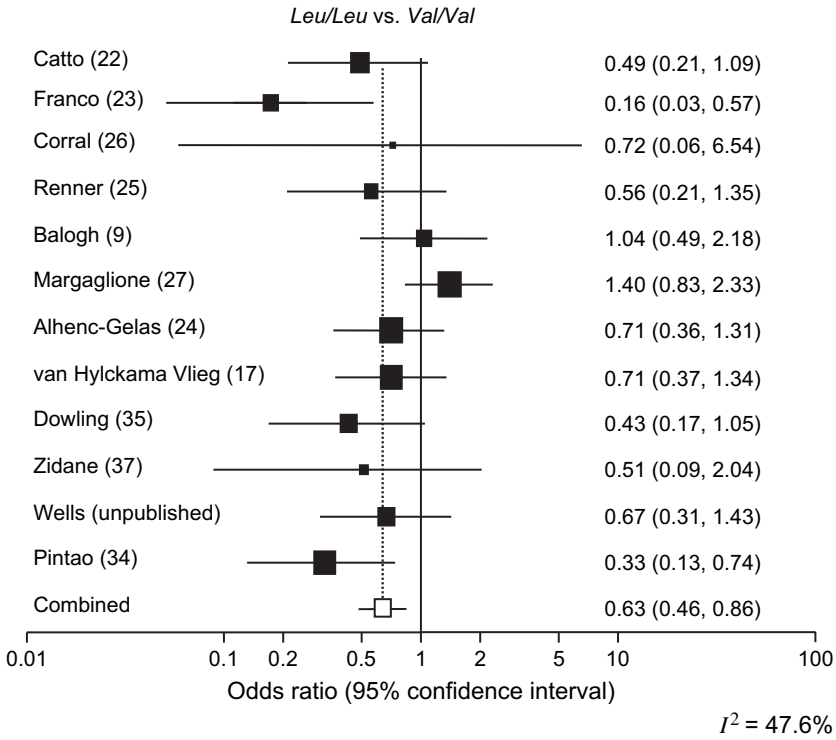

FIGURE 1. Odds ratio (95% confidence interval) for the association between the *Leu/Leu* genotype vs. the *Val/Val* genotype of the *FXIIIA* gene and venous thromboembolism. On the left, the first author of the study is followed by the reference number in parentheses. The size of the black box corresponding to each study is proportional to the sample size; the horizontal lines show the 95% confidence interval of the odds ratio. The combined estimate is based on a random-effects model shown by the dashed vertical line and white box. The solid vertical line represents the null result: an odds ratio of 1. The I^2 statistic is shown in the bottom right corner.

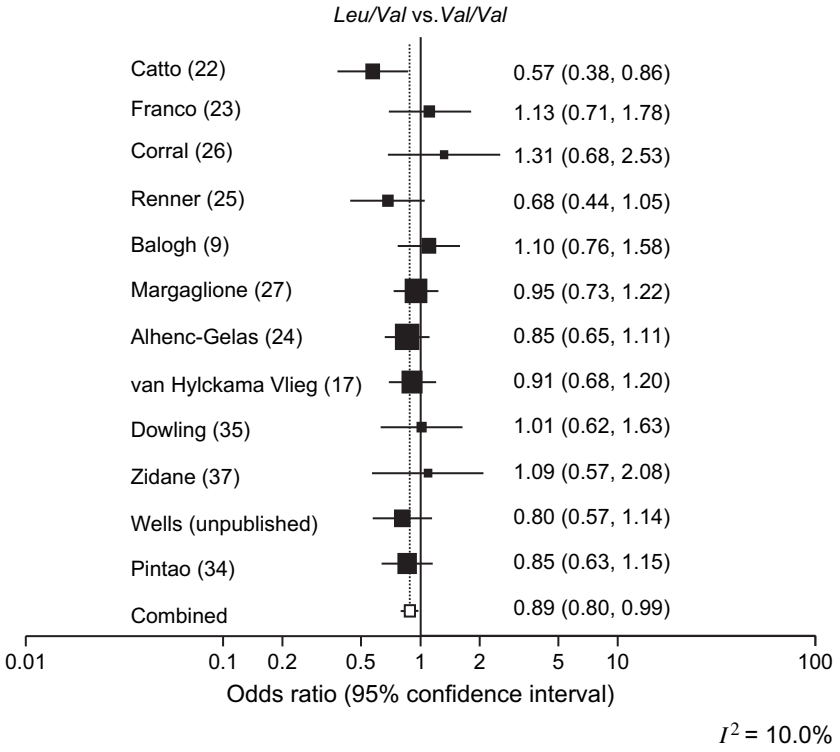

FIGURE 2. Odds ratio (95% confidence interval) for the association between the *Leu/Val* genotype vs. the *Val/Val* genotype of the *FXIIIA* gene and venous thromboembolism. On the left, the first author of the study is followed by the reference number in parentheses. The size of the black box corresponding to each study is proportional to the sample size; the horizontal lines show the 95% confidence interval of the odds ratio. The combined estimate is based on a random-effects model shown by the dashed vertical line and white box. The solid vertical line represents the null result: an odds ratio of 1. The I^2 statistic is shown in the bottom right corner.

when the four studies with selection criteria likely to bias the results were excluded from the analysis, leading to even stronger protective effects of the *FXIII Val34Leu* variant against VTE and somewhat narrower confidence intervals.

Our meta-analysis included an additional 983 cases and 1,135 controls compared with the largest of the two meta-analyses of studies examining the *FXIII Val34Leu* variant in relation to VTE (17, 24). The meta-analysis by Alhenc-Gelas et al. (24) combined the results of five studies (1,340 cases and 2,211 controls) and found a statistically significant protective effect of the *FXIII Val34Leu* variant against VTE, with odds ratios of 0.58 (95 percent CI: 0.41, 0.82) for homozygotes (*Leu/Leu*), 0.86 (95 percent CI: 0.74, 0.99) for heterozygotes (*Leu/Val*), and 0.80 (95 percent CI: 0.69, 0.94) for homozygotes and heterozygotes combined (*Leu/Leu* + *Leu/Val*). The meta-analysis by van Hylckama Vlieg et al. (17), which included three additional studies, reported a smaller, nonstatistically significant effect despite an additional 842 cases and 1,563 controls compared with the meta-analysis by Alhenc-Gelas et al. In the meta-analysis by van Hylckama Vlieg et al., the odds ratios for the homozygotes were 0.80 (95 percent CI: 0.60, 1.00), 0.80 (95 percent CI: 0.74, 0.99) for the heterozygotes, and 0.90 (95 percent CI: 0.80, 1.00) for the homozygotes and heterozygotes combined. These authors also analyzed their data by excluding two of the new studies in which cases were further ascertained for known thrombophilias (i.e., Balogh et al. (9) and Margaglione et al. (27)). When these studies were excluded from the analysis, the effects became more prominent and statistically significant, with odds ratios of 0.60 (95 percent CI: 0.40, 0.80) for the homozygotes, 0.80 (95 percent CI: 0.70, 1.00) for the heterozygotes, and 0.80 (95 percent CI: 0.70, 0.90) for the homozygotes and heterozygotes combined. The Margaglione et al. cases were all derived from referral for thrombophilic workup; therefore, the most critically ill subjects may have been referred. In the Balogh et al. study, the cases had their first thrombotic episode at an unusually young age; other inclusion criteria were positive family history of VTE or recurrent thrombosis or thrombosis at unusual sites.

These observations suggest that the two studies may have selected cases with more than one determinant for VTE, including additional genetic determinants, which introduced heterogeneity, possibly biasing the estimated odds ratios. It was not possible to specifically assess the risk of VTE in this subgroup—that is, cases with a known predisposition to VTE—because the two studies did not follow the same ascertainment scheme. For example, the mean age of cases in the Balogh et al. study (9) seemed to be younger than that in the Margaglione et al. study (27). However, the age comparison is not simple here. Balogh et al. presented the mean age of cases, whereas Margaglione et al. presented the

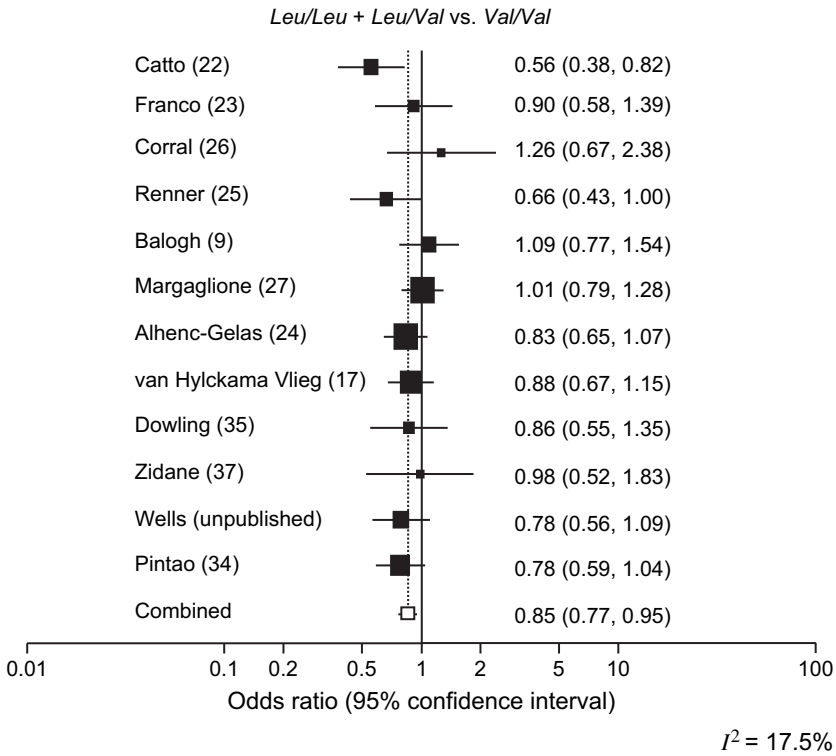

FIGURE 3. Odds ratio (95% confidence interval) for the association between the *Leu/Leu* + *Leu/Val* genotypes combined vs. the *Val/Val* genotype of the *FXIIIA* gene and venous thromboembolism. On the left, the first author of the study is followed by the reference number in parentheses. The size of the black box corresponding to each study is proportional to the sample size; the horizontal lines show the 95% confidence interval of the odds ratio. The combined estimate is based on a random-effects model shown by the dashed vertical line and white box. The solid vertical line represents the null result: an odds ratio of 1. The I^2 statistic is shown in the bottom right corner.

median age. This problem, along with the small sample size of the subset, did not allow subgroup analysis.

Potential problems in conducting and reporting genetic association studies of complex traits have been well described by several authors (38–41). One such problem, population stratification, is always a concern in genetic association studies that use data on unrelated persons. However, to minimize the risk of this potential confounder, in a subgroup analysis we included data samples predominantly composed of White subjects. Work by Ioannidis et al. (42) suggests that such an approach is likely to be sufficient to avoid confounding effects due to population structure and that indeed genetic variants vary in frequency across populations, but the biologic impact usually is consistent across racial boundaries. We found no change in our results with this subgroup analysis. The absence of deviation from Hardy-Weinberg equilibrium of almost all individual studies included in the meta-analysis supports the absence of population stratification. Hardy-Weinberg equilibrium is also consistent with a good selection of genomic controls.

Potential problems in applying meta-analysis methods are also of concern (29, 43). We made attempts to deal with them in the current study. Our meta-analysis used methods and statistical significance thresholds that provided conservative estimates. We did not detect publication bias or heterogeneity between studies. Since we did not have access to individual subjects' data that would have enabled us to use an approach based on individuals, heterogeneity may have remained masked. We identified two potential sources of heterogeneity by carefully inspecting the selection criteria of the individual studies selected for our meta-analysis. The two studies identified as a potential source of heterogeneity were those previously identified by van Hylckama Vlieg et al. (17). We reanalyzed the data by excluding the two studies and the study not in Hardy-Weinberg equilibrium. Doing so did not change our conclusion; rather, their exclusion increased the protective effect measured for the *FXIII Val34Leu* variant against VTE. The odds ratios, particularly for the homozygotes, were lower and the confidence intervals were narrower.

Of particular note here is the vague description of the ethnic background of the study sample and the limited information on the procedures related to determining the genotypes in the vast majority of studies. Several studies did not describe the ethnic background of their samples in percentages or absolute values. Moreover, although most studies referred to a polymerase chain reaction approach for genotyping, only about half of the published studies specifically named the primers used (9, 17, 23, 26, 37), two others referred to a previous paper, and the remainder did not mention anything regarding the primers used. Only two studies mentioned whether the laboratory technicians were blinded

to the case/control status of the samples. Genotyping error rates were not mentioned in any of the studies. Although we acknowledge that the genotyping error rate may be difficult to estimate in the context of biallelic markers, such as for the *FXIII Val34Leu* variant and in the absence of parental data, more attention to this and other information related to the genotyping section of the methodology would be useful in comparing the studies and in evaluating their quality.

Another problem that complicates the comparison of genetic association studies, and the pooling of data for meta-analysis purposes, is the nonstandardized definition of cases. For example, the majority of studies ascertained the cases with either first or recurrent VTE, and it was not noted whether VTE was secondary to transient risk factors. Furthermore, in several studies, patients with cancer were also included. It is well known that cancer represents a hypercoagulable state. Conversely, experiencing VTE secondary to transient risk factors is associated with low risk for recurrence and has not been well linked to genetic predisposition. Since the data from individual studies were not presented in a way that enabled this type of subgroup analysis, the implications of these factors could not be verified in our analysis. Ideally, future association studies, either on *factor XIII Val34Leu* or other variants, should be more specific regarding the phenotype definition.

The implication of the *FXIII Val34Leu* variant as a factor conferring protection against VTE is biologically plausible. It has been demonstrated that, in carriers of the *FXIII Val34Leu* variant, activation and depletion of the plasma FXIIIA subunits is more rapid (2). This action results in less stable clots and indeed may result in a decrease in FXIIIA available for stabilization of the clot and thus could provide a protective effect against VTE. VTE, as other complex and multifactorial diseases are, is likely influenced by several genes as well as environmental factors. Simultaneously considering genetic and nongenetic risk and protective factors will be necessary to develop accurate risk profiles to identify persons at risk of VTE. For example, Lim et al. (44) suggest that the protective effect of the *FXIII Val34Leu* variant is specific to conditions in which fibrinogen levels are high, that is, levels known to be associated with an increased risk of VTE. At higher fibrinogen levels, clots in *Leu/Leu* persons were more permeable and looser than in *Val/Val* persons, characteristics associated with more breakable clots. Similar changes in permeability were also observed for a *fibrinogen* variant (44). Thus, the joint effect of genetic variants and hemostatic trait concentrations is likely to be important in predicting the risk of VTE. This is only one example of the complexity likely to be underlying venous thromboembolic disorders. While it may be premature to routinely test for the *FXIII Val34Leu* variant, our results suggest that this variant will be a useful component of risk profiles in the future.

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